

# Influence of Mobile Phone Radiation on Membrane Permeability and Chromatin State of Human Buccal Epithelium Cells

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## Abstract

The results obtained in the present study indicate a statistically significant response of human buccal epithelium cells to the mobile phone radiation in terms of changes in the number of heterochromatin granules in nucleus and membrane permeability. The observed changes are qualitatively similar, indicating certain threshold exposure time of about 1 min and saturation at exposure times longer than 30 min. These changes may serve as a marker of alteration in functional activity of human cells after electromagnetic radiation (EMR) action. The major effect of mobile phone radiation on cells is dependent upon the exposure time, whereas the specific absorption rate (SAR) level of mobile phone radiation appears to be relatively unimportant. As our experiments are conducted using limited number of subjects, the data obtained in this study are preliminary and more extensive studies are needed.

## Keywords

*Buccal Epithelium Cell; Heterochromatin; Indigo Carmine; Membrane Permeability; Mobile Phone; Orcein*

## Introduction

Throughout his life a human being is constantly exposed to electromagnetic radiation (EMR) of artificial origin with different frequencies and power. One of the major types of EMR which we are dealing with on a daily basis is microwave radiation used by modern means of communication such as mobile phones, Bluetooth, WiFi, WiMAX etc.

Previously, several authors have studied the effects of the radio frequency (RF) radiation generated by mobile phones on living cells of different organisms, and in a number of studies a pronounced negative effect of different nature has been demonstrated, for

example (Tice et al., 2002; Salford et al., 2003; Diem et al. 2005; Balci et al., 2007). These results suggest that the low-intensity microwave radiation can induce a statistically-significant response at cellular level. Contrary to that, a number of studies have not reported any negative effect of microwave radiation (Capri et al., 2004; Komatsubara et al., 2005).

Recently (Shckorbatov et al., 2009) a program of directed search of a relation between various parameters of RF electromagnetic field and cell response has been initiated using human buccal epithelium cells as an object of investigation. In particular, it was shown that low-intensity microwave radiation causes an increase in heterochromatin granule quantity (HGQ) and leads to condensation of chromatin in buccal cell nuclei. The HGQ parameter was also shown to be sensitive to the action of other factors such as biologically active compounds, UV and laser irradiation (Shckorbatov et al., 1998, 2001, 2002, 2009). The effect of chromatin condensation was also demonstrated in human lymphocytes in the studies (Sarimov et al., 2004; Markova et al., 2005) with the parameters of electromagnetic field similar to the radiation of mobile phones. These results suggest that the state of chromatin may serve as an indicator of sensitivity of human cells to the action of EMR, however, it does not necessarily mean that the functional activity of human cells alters. If, however, the chromatin granulation reflects the cell reaction to EMR and is linked to a change in chromatin functional activity, it may be expected that the response to EMR may also be observed in other cellular components. It is known that an important role in the reception of microwave radiation is played by cellular membrane,

the state of which is linked with the state of the cell itself (Shckorbatov et al., 1995; Pasiuga et al., 2009).

In order to deepen our understanding of the influence of EMR on living cells, we have performed *in vitro* investigation of the chromatin condensation and membrane permeability changes in human buccal epithelium cell upon irradiation by electromagnetic field of mobile phones.

## Materials and methods

### Human Cells

Experiments were conducted on short-term cell culture of human buccal epithelium. Cells were obtained from the inner surface of the donor cheek by scraping with a blunt sterile spatula. This procedure is absolutely bloodless and painless and all donors have been pre-informed about the aims of the study. Cells were placed in 3.03 mM phosphate buffer (pH 7.0) supplemented with 2.89 mM calcium chloride. It was shown previously that if cells are placed in a solution of such composition for 24 hours, no visible changes in the structure of the nucleus and cell membrane could be detected (Shckorbatov et al., 1998). After that the solution with the suspended cells was subjected to EMR of mobile phone of definite duration.

Cells were collected from three male donors of different ages: A - 35, B - 25, C - 24 years old, and one female donor: D - 22 years. All the donors were not smokers.

### Selection of the Mobile Phones as the Sources of EMR

Mobile phones currently produced worldwide differ in the irradiated power. A parameter SAR used as a characteristic of a mobile phone has a meaning of a power of electromagnetic field absorbed by biological tissue per unit of mass. SAR can alternatively be considered as a measure of interaction between EMR and biological object that can indirectly affect the effect of mobile phones in respect to a human being.

In order to make a selection of mobile phones with most common SAR levels, a statistical distribution of SAR has been built based on official data for most popular mobile phones brands (Nokia, Samsung, Sony Ericson, Motorola, LG, Siemens, Sony etc), taken from the official websites of their manufacturers. It was shown (see Supplementary material) that the most widely used mobile phones correspond to SAR values in the range between 0.5 to 1.1 W / kg. As a source of

EMR, two mobile phones were selected corresponding to the edges of this distribution with  $SAR_1=0.531$  W/kg and  $SAR_2=1.1$  W/kg and a frequency of 900 MHz, additionally allowing for the consideration on whether the irradiated power of the mobile phone has any effect on human cells. In all experiments, the mobile phones were used in talk mode. Although it is acknowledged that the SAR level and the actual power of mobile phone during the experiment are likely different, our results, however, indicate relative biological effect which is proportional to SAR taking into account that both mobile phones are placed at the same location and operated at the same phone network.

### Irradiation Procedure

The solution containing the cells and phosphate buffer was distributed among 0.5 ml Eppendorf micro test tubes which were then placed to a Petri dish. Mobile phone was located above the test tubes at a distance of 3-4 cm. The exposure time was 1, 2, 5, 10, 15, 30 and 60 minutes, covering the most probable durations of using the mobile phones in a talk mode. Each experiment for each donor was carried out within one day at room temperature and daylight. Control sample for each donor collected at the same time at each treatment was incubated under similar conditions as in irradiated samples. No signs of cell degradation during the experiment were observed, as evidenced by the absence of changes in cell morphology and constancy of the measured parameters in the control sample over time.

### Chromatin State Evaluation

The functional state of cell nucleus is directly related to the structural transitions from heterochromatin to euchromatin. Increase in HGQ parameter indicates a lower transcription activity in the nucleus. Estimation of HGQ parameter was made by the method proposed in (Shckorbatov et al. 2009). Irradiated cells and the control sample were stained with 2% orcein solution in 45% acetic acid (Shckorbatov et al., 1999). Cell nuclei were visually examined by means of a microscope MICROMed XS-3330 with magnification of 1000 (Fig. 1).

In each experiment, the HGQ parameter was determined in 30 cell nuclei. It was previously shown (Shckorbatov et al. 2009) that such number of the examined nuclei is optimal, i.e. further increment in the number of analyzed nuclei does not result in significant decrease of standard error, but remarkably

slows down the whole analysis. The scorer of the HGQ was blind to the exposure conditions and all measures were made by one person.

In all experiments, the mean value of the HGQ was determined. As a main quantity representing the chromatin reaction to EMR, we used a difference between the HGQ of the irradiated sample and HGQ of the control normalized to the HGQ of the control, i.e. a relative change in a quantity of granules of heterochromatin or the relative quantity index (RQI):

$$RQI = \frac{HGQ - HGQ_{control}}{HGQ_{control}}$$

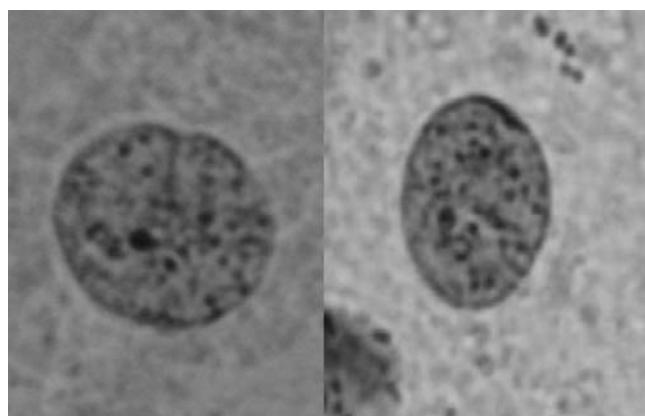


FIG. 1 THE NUCLEUS OF HUMAN BUCCAL EPITHELIUM CELL BEFORE (LEFT) AND AFTER (RIGHT) IRRADIATION

### Membrane State Evaluation

In order to determine the change in permeability of the cellular membranes under the influence of EMR, the irradiated cells were stained with vital dye indigo carmine. It is considered (Shckorbatov et al., 1995) that the percentage of the cells stained with this dye may serve as an indicator of membrane integrity.

The state of cell membranes was assessed by the percentage of cells stained *in vitro* with 5 mM solution of indigo carmine within one minute (an example of stained cells is given in Fig. 2) as recommended in (Shckorbatov et al., 1995).

In the course of determination of the percentage of stained cells (PSC, %), in each experiment 300 cells (3 repeats by 100 cells) were examined. The counting of stained cells was done by person blind to the exposure conditions.

Counting of stained cells was accomplished by means of a microscope MICROmed XS-3330 with magnification of 400, and the mean value of PSC was determined. As a main quantity, representing the cell membrane reaction to EMR, we used a difference

between the PSC of the irradiated sample and PSC of the control normalized to the PSC of the control, i.e. a relative change in the number of stained cells, or relative staining index (RSI):

$$RSI = \frac{PSC - PSC_{control}}{PSC_{control}}$$

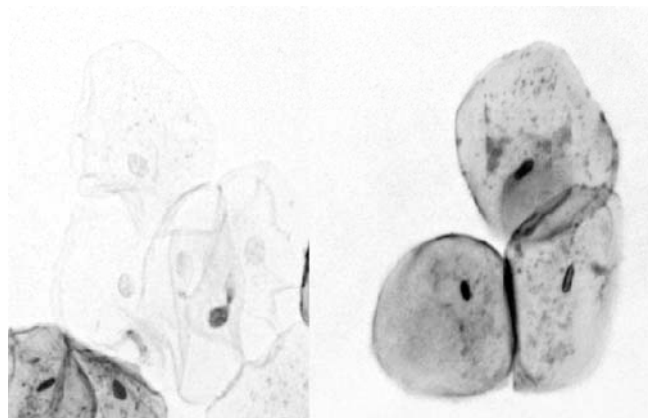


FIG. 2. THE HUMAN BUCCAL EPITHELIUM CELLS NOT STAINED (LEFT) AND STAINED (RIGHT) BY INDIGO CARMINE

### Statistical Analysis

All computations of means and standard errors were made in Microsoft Office Excel. Statistical significance of all observed differences between the means of HGQ and PSC and control values was evaluated using Student's t-test. In order to assess the statistical relationship between the obtained data and the influence of various factors, the analysis of variance ANOVA was performed in Statistics6 software. In all cases, the confidence level was taken as  $P=0.05$ .

### Results

#### Effect of Mobile Phone Radiation on Chromatin State in the Buccal Epithelium Cell Nuclei

The results of the influence of mobile phone radiation on chromatin state in buccal epithelium cell nuclei are shown in Fig. 3 for donors A-D (the numerical values are provided in Supplementary material). As it can be seen that the reaction of RQI to the mobile phone irradiation was observed in cells of all tested donors, so the mobile phone radiation was induced in cells in the process of chromatin condensation. Even at a relatively short exposure time of 1 minute, the irradiation produced the significant RQI level in cells of donors A, B, and D. The increase of exposure time from 1 to 10 minutes resulted in the further increase of RQI. In cells of donor C, only radiation produced by the mobile phone with a high characteristic of SAR induced a significant RQI at exposure times of 1, 2 and

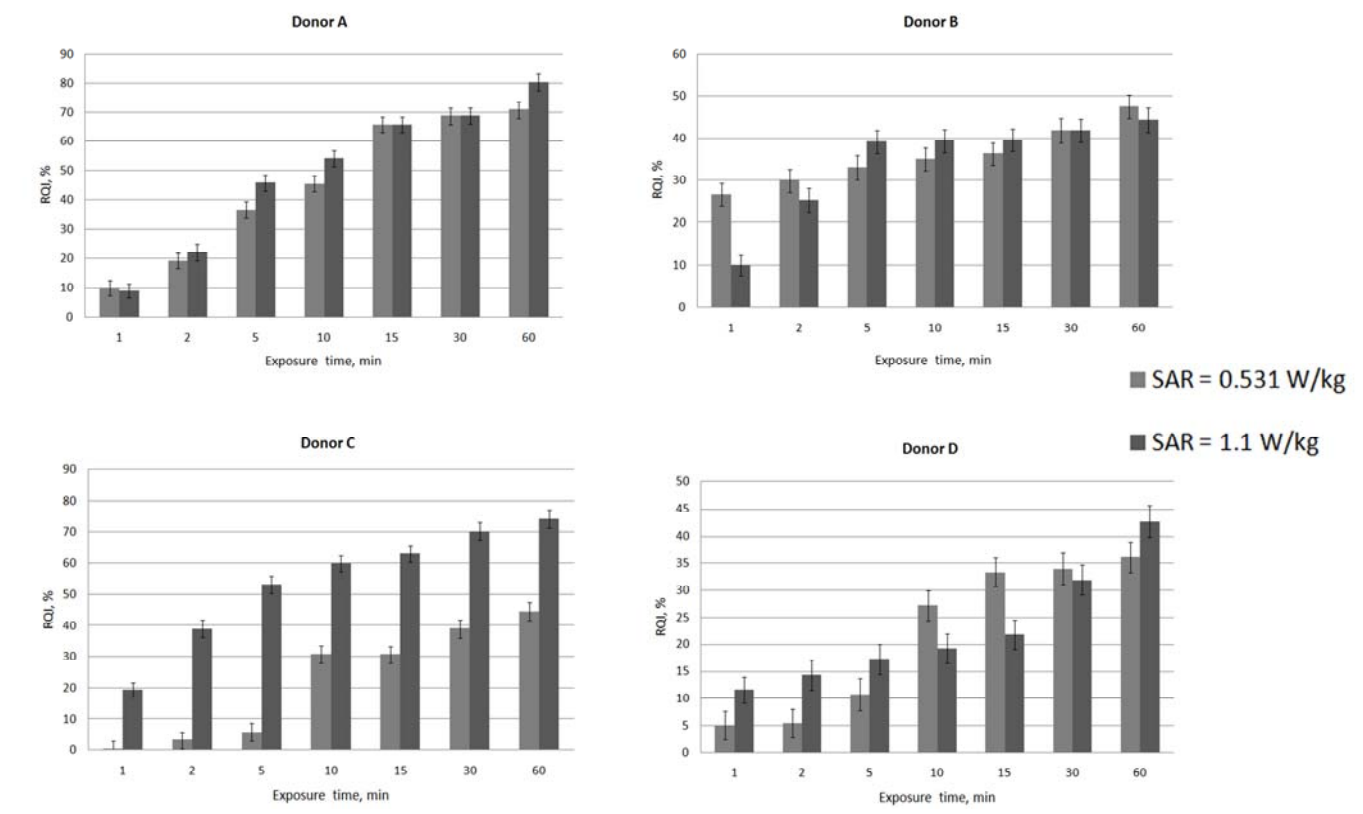


FIG. 3. RELATIVE CHANGE IN THE NUMBER OF GRANULES OF HETEROCHROMATIN (RQI) AS A RESULT OF CELLS' EXPOSURE TO MOBILE PHONE IRRADIATION (DATA FOR FOUR DONORS, RELATIVE QUANTITY INDEX  $\pm$  SEM, IN EACH EXPERIMENT N=30)

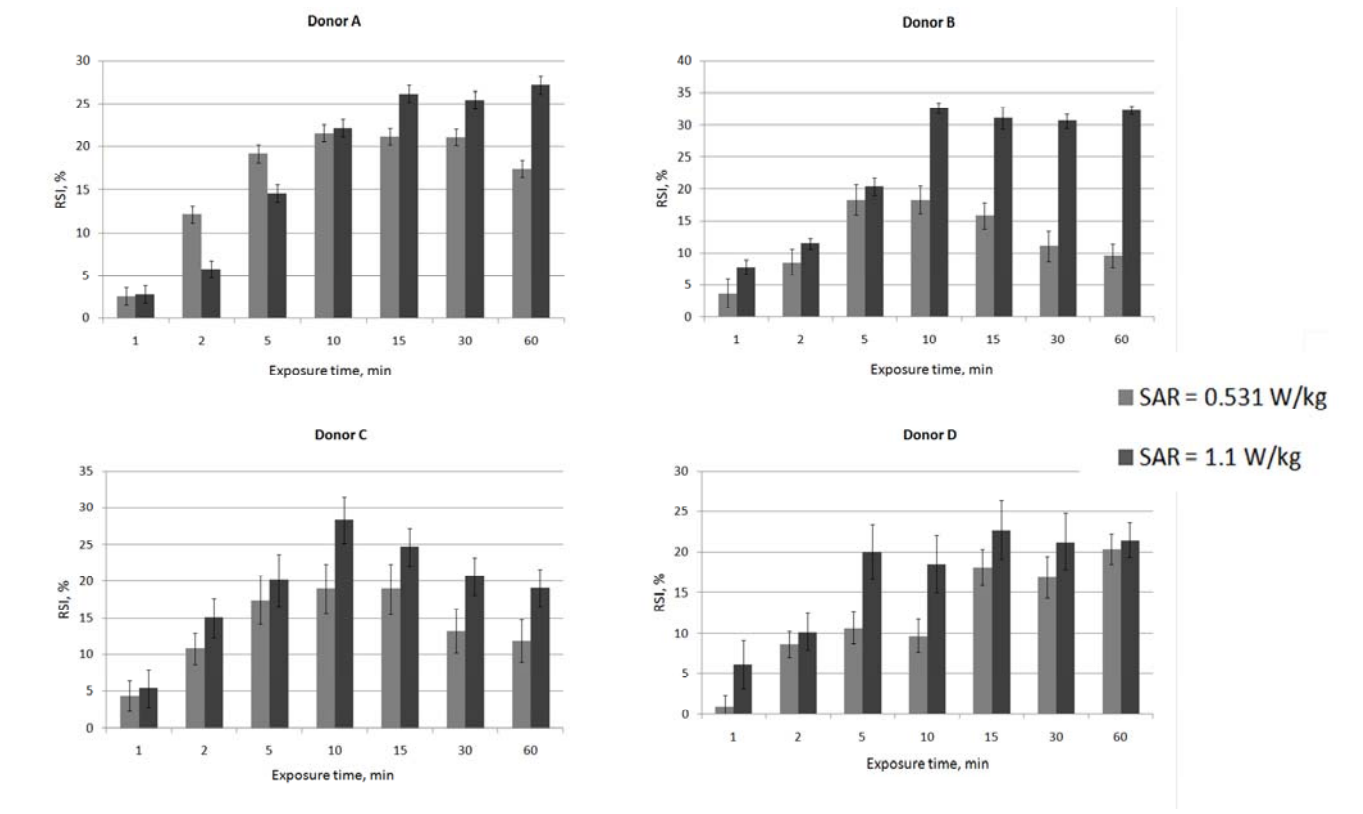


FIG. 4. RELATIVE CHANGE IN CELLULAR MEMBRANE PERMEABILITY (RSI) AS A RESULT OF CELLS' EXPOSURE TO MOBILE PHONE IRRADIATION (DATA FOR FOUR DONORS, RELATIVE STAINING INDEX  $\pm$  SEM, IN EACH EXPERIMENT N=3)

5 minutes. These results indicate that for different donors there are individual traits in their cell reaction to mobile phone radiation. In cells from donor C, the radiation of the mobile phone with declared higher SAR induced more effect than that with lower declared SAR. Interestingly, in cells of Donor B at the low exposure time (1 minute), the radiation of the mobile phone with a low SAR produced more effect than radiation of the mobile phone with higher SAR.

The same regularity was observed in cells of Donor D at exposure times of 10 and 15 minutes. In cells of Donors A and B, the irradiation by the mobile phones with both high and low declared SAR produced the same cell effect at exposure time from 2 to 60 minutes. The key result here is that the mobile phone radiation with different SAR levels leads to statistically significant increase in RQI value for all donors studied, starting from the exposure time longer than 1 minute. Similar effect for buccal epithelium cells was observed in the studies (Shckorbatov et al., 2002, 2009) with low-intensity microwave radiation of different polarization.

#### ***Effect of Mobile Phone Radiation on Membrane Permeability of the Buccal Epithelium Cells***

The results of the experiments on the cell membrane response to mobile phone EMR are presented in Fig. 4 for donors A-D (the numerical values are provided in Supplementary material). As one can see from the data, a statistically significant response of the cells to electromagnetic radiation with various exposure time is observed and expressed in the increase of membrane permeability relatively to the control (RSI parameter). As a rule, the membrane permeability increased with the rise of exposure time from 1 to 10 minutes (like a RQI parameter). The further increase of exposure time from 10 to 60 minutes resulted in decrease of RSI in cells of Donor C for both variants of irradiation, and in cells of Donor B only for irradiation by mobile phone with a low declared SAR. As for the cells from other donors, the prolongation of exposure time beyond 10 min had almost no effect on RSI. The irradiation by mobile phones with more declared SAR induced more effect on cell membranes in cells from donors A, B, and C at exposure time from 10 to 60 minutes.

#### **Discussion**

##### ***Effect of Mobile Phone Radiation on Chromatin State in the Buccal Epithelium Cell Nuclei***

For each donor and SAR, a positive correlation between the RQI parameter and the exposure time has

been observed, *viz.* an increase in exposure leads to increase in chromatin granulation. However, no statistically meaningful differences in RQI parameter for different SAR levels have been observed for donors A, B and D, whereas for donor C a higher level of SAR leads to more rapid and intense growth of RQI, which can be explained by individual differences in donor cells susceptibility to EMR. The t-test showed that in all cases studied the relative change in the number of heterochromatin granules is statistically significant for the exposure times equal or higher than 1 min.

Hence the threshold exposure time when the structure of heterochromatin visibly changes under the influence of EMR of mobile phone is below 1 min.

Based on the results obtained above, it is possible to state that the condensation of chromatin (change in RQI parameter) may act as a marker of cell response to the action of EMR. It is thought that this effect can be due to changes in DNA-protein interactions caused by radiation (George et al., 1987), and may lead to mutations (Surrals et al., 1998). It is also important to note that according to the current knowledge the formation of heterochromatin is associated with a decrease of transcriptional activity of chromatin (Prokofieva-Bel'govskaya, 1945). Therefore, the EMR, investigated in the present study, changes the functional activity of cells, which is manifested by the increase in a number of heterochromatin granules.

##### ***Effect of Mobile Phone Radiation on Membrane Permeability of the Buccal Epithelium Cells***

It is seen that the behavior of membrane permeability as a function of the exposure time is different from that observed for the granulation of heterochromatin, *viz.* for the majority of donors at times less than 10 min an increase in RSI is observed, followed by a peak around 10 min exposure time and then, a decrement of RSI. For the mobile phone with higher SAR (*i.e.* SAR<sub>2</sub>) the peak is not observed for the donors A, B and D which is, probably, due to a finite exposure time of 60 minutes used in experiments.

The t-test has shown that for all donors and all SAR levels the threshold exposure time exceeds 1 min during which no statistically significant increase in RSI is observed.

For all donors and SAR levels, the change in membrane permeability during exposure with durations from 30 to 60 min was not statistically

significant. This observation can be interpreted as adaptation of the cells to EMR action, which may be associated with effect of restoration of cellular membranes after exposure to EMR (Pasiuga et al., 2009). It is reasonable to assume that the peak in membrane reaction to EMR for low SAR level observed in Fig. 4 is also a consequence of this recovery effect.

Analysis of the RSI parameter as a function of SAR of the mobile phone used for each donor has shown that for the majority of donors and exposure times there is a statistically significant difference between the RSI values for the mobile phones with different SARs, i.e. the increment of power (increase of SAR) leads to the increase in membrane permeability. It is, however, important to note that, according to the obtained results, the dependence of cell response to EMR of mobile phones in terms of the membrane permeability has appeared as unapparent, i.e. the change of HGQ is not proportional to SAR for the cells from Donor D, and in case of certain exposure times for the rest of the donors it is statistically not significant or even reversed. It means that for the most popular mobile phone brands used worldwide the selection of mobile phones with different radiation power is not an important factor in terms of buccal epithelium cells response.

By means of comparison between the buccal epithelium cells response to EMR of mobile phones discussed above in the context of heterochromatin granulation in nucleus (RQI parameter) and permeability of cellular membrane (RSI parameter), one can conclude that within the same power levels and exposure times of irradiation, the RQI and RSI parameters demonstrate statistically significant and qualitatively similar changes, manifesting the certain threshold exposure time and saturation in time. These changes may serve as a marker of alteration in functional activity of human cells as a result of EMR action, as well as a single mechanism of cell reaction to EMR, which remains currently unknown. Currently some ideas on the molecular mechanisms of microwave EMR influence on living organisms have been suggested, including the polarization of bound charges, the orientation of permanent dipoles and the movement of free ions (Bernhardt, 1992; McKinlay and Bernhardt, 2003), which may give contribution to the observed effects in human buccal epithelium cells.

#### ANOVA analysis

In order to assess the influence of various factors, such as SAR, exposure time and individual differences in

donors, analysis of variance (ANOVA) was carried out (Table 1). It is seen that the SAR of mobile phone and the exposure time have the most significant effect on the chromatin state. It is, however, different from the cell membrane reaction, since the greatest influence on the cell membrane permeability is noted for the 'Donor' factor. It is likely that due to a limited number of SAR levels and donors used in our analysis, the results for F criterion for 'SAR' and 'Donor' factors are approximate, whereas the changes in HGQ and PSC with the 'Time' factor are more reliable.

TABLE 1 ANOVA ANALYSIS OF BUCCAL EPITHELIUM CELLS RESPONSE TO EMR OF THE MOBILE PHONES

Factor	F	P
The effect of mobile phone radiation on chromatin		
SAR	<b>875,1</b>	<0.001
Time	767,6	<0.001
SAR*Time	13,7	<0.001
Donor	240,2	<0.001
Donor*SAR	618,5	<0.001
Donor*Time	28,9	<0.001
Donor*SAR*Time	11,7	<0.001
The effect of mobile phone radiation on membrane permeability		
SAR	267,3	<0.001
Time	183,3	<0.001
SAR*Time	9,4	<0.001
Donor	<b>847,9</b>	<0.001
Donor*SAR	122,8	<0.001
Donor*Time	6,4	<0.001
Donor*SAR*Time	2,3	<0.001

Notes: F – Fisher's criterion, P – significance level.

#### Conclusions

The results obtained in the present study indicate a statistically significant response of human buccal epithelium cells to the mobile phone radiation in terms of changes in the number of heterochromatin granules in nucleus and membrane permeability. The observed changes are qualitatively similar, indicating certain threshold exposure time of about 1 min and saturation at exposure times longer than 30 min. These changes may serve as a marker of alteration in functional activity of human cells after EMR action. Duration of the exposure has the greatest influence on the cell response to mobile phone EMR, whereas the SAR level of mobile phone between 0.53 and 1.1 W/kg appears to be relatively unimportant. As our experiments have

been conducted using limited number of subjects, the data obtained in this study are preliminary and more extensive studies are needed.

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